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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Girard et al.
Appl. No.	:	10/601,072
Filed	:	June 19, 2003
For	:	CHEMOKINE-BINDING PROTEIN AND METHODS OF USE
Examiner	:	Lei Yao
Group Art Unit	:	1642

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. JEAN-PHILIPPE GIRARD UDNER 37 C.F.R. § 1.132

Sir:

I, Dr. Jean-Philippe Girard, do hereby declare and state that:

1. I am a co-inventor of the subject matter described and claimed in U.S. Patent Application Serial No. 10/601,072, filed on June 19, 2003 entitled, "CHEMOKINE BINDING PROTEIN AND METHODS OF USE."

2. I am the Director of the Department of Cancer Biology and the Head of the Laboratory of Vascular Biology at the Institut de Pharmacologie et de Biologie Structurale, Toulouse, France. My curriculum vitae is attached as Exhibit A.

3. I am a co-founder and part-owner of Endocube SAS, which is the assignee of the instant patent application.

4. I have read and understand the specification and claims of United States Patent Application No. 10/601,072. I am familiar with the prosecution history of this application and I understand that, in the response to the Office Action issued April 23, 2007, independent claim 15 will be amended as set forth below:

Claim 15. A method of inhibiting the activity of a chemokine, said method comprising contacting a chemokine with an agent comprising a polypeptide selected from the group consisting of SEQ ID NO: 3, a polypeptide having at least 95% sequence identity to SEQ ID NO: 3, a chemokine-binding domain of SEQ ID NO: 3 and a polypeptide having at least 95% sequence identity to a chemokine-binding domain of SEQ ID NO: 3, wherein the activity of said chemokine is inhibited.

5. I understand the Examiner rejected claim 15 and the claims dependent thereon as not being adequately enabled by the specification as filed. It is also my understanding that during a telephonic interview of October 18, 2007, the Examiner and her supervisor indicated that a declaration should be provided, which contains data showing that contacting a chemokine with a chemokine-binding domain of SEQ ID NO: 3 inhibits chemokine activity.

6. I declare that, either directly or under my supervision, experiments were performed which demonstrate that providing a chemokine-binding domain of SEQ ID NO: 3, both *in vitro* and *in vivo*, results in an inhibition of chemokine activity as recited in independent claim 15. These experiments are described in certain examples set forth in co-pending, co-owned United States Patent Application No. 11/360,450, an application for which I am a co-inventor.

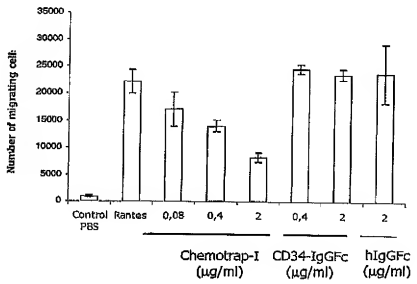
7. In the first experiment, which is described in Example 41 of United States Patent Application No. 11/360,450, we demonstrated that the chemokine binding domain of SEQ ID NO: 3 (THAP1 chemokine-binding domain) fused to the constant region of immunoglobulin G (IgG1-Fc) possessed the ability to inhibit white blood cell chemotaxis mediated by chemokine CCL5 (Rantes) *in vitro*. The experimental procedure and results set forth in Example 41 of United States Patent Application No. 11/360,450 are reproduced as follows:

> In this Example, we demonstrate the ability of a THAP1 chemokine-binding domain/IgG1-Fc fusion constructed as described in Example 18B to inhibit white blood cell chemotaxis *in vitro* mediated by Rantes/CCL5 in a human monocyte cell line expressing the Rantes/CCL5 receptor, CCR3. Control vehicles were generated as fusion proteins between the C-terminal domain of the CD34 glycoprotein and the human IgG1-Fc region polypeptide. Thp1 cells were obtained from ATCC (Acc. TIB-202) and grown in DMEM medium according to provider's instructions. Recombinant Rantes/CCL5 human chemokine was obtained from commercial suppliers (for example, R&D or Chemicon).
>
> Chemotaxis assays were setup using a 96-well two chamber system. First, the appropriate culture medium was added to wells of a 96-well microtiter plate. Recombinant chemokine Rantes/CCL5 was diluted to 80ng/ml in the appropriate culture medium and 15µl of this solution were then transferred to the wells of the 96-well microtiter plate. Both the THAP1 chemokine-binding domain IgG1-Fc fusion and the control fusion were serially diluted starting at 500nM and then 15µl

of each were transferred into appropriate wells. Subsequent to the addition of the chemokine and fusions, 96-transwell chambers were set carefully onto the plate and 100µl of a monocyte cell suspension containing 1.107cell/ml were added in the transwell (upper) chambers. Following a 2 hour incubation at 37°C and 5% CO_2, cell migration to the lower chambers was quantified by using the luminescent Celltiter Glo system (Promega). The level and specificity of Rantes/CCL5-induced chemotaxis inhibition by the THAP1 chemokine-binding domain/IgG1-Fc fusion was determined by comparing the number of cells migrating in the presence of this fusion to cells migrating in the presence of the control fusion.

Figure 24 shows that when Rantes/CCL5 alone was supplied to the moncytes, over 22,000 migrating cells were observed. Similar results were seen when the monocytes were incubated in the presence of Rantes/CCL5 and human IgGFc (2 µg/ml) or Rantes/CCL5 and CD34/IgGFc fusions (at 0.4 µg/ml and 2 µg/ml). However, when monocytes were incubated in the presence of Rantes/CCL5 and the THAP1 chemokine-binding domain/IgG1-Fc fusion, the number of migrating cells were significantly decreased. When the THAP1 chemokine-binding domain/IgG1-Fc fusion was supplied at 0.08 µg/ml the number of migrating monocytes decreased to about 17,000. When the THAP1 chemokine-binding domain/IgG1-Fc fusion was supplied at 0.4 µg/ml the number of migrating monocytes decreased to about 14,000. Finally, when the THAP1 chemokine-binding domain/IgG1-Fc fusion was supplied at 2.0 µg/ml the number of migrating monocytes decreased to less than 8000.

FIGURE 24



8. In the second experiment, which is described in Example 42 of United States Patent Application No. 11/360,450, we demonstrated that the chemokine binding domain of SEQ ID NO: 3 (THAP1 chemokine-binding domain) fused to the constant region of immunoglobulin G (IgG1-Fc) possessed the ability to inhibit chemokine-induced cell recruitment mediated by CCL5 (Rantes) and to inhibit chemokine-induced cell recruitment mediated by CCL1 *in vivo*. The experimental procedure and results set forth in Example 42 of United States Patent Application No. 11/360,450 are reproduced as follows:

> In this Example, we demonstrate the ability of a THAP1 chemokine-binding domain/IgG1-Fc fusion constructed as described in Example 18B to inhibit chemokine-induced cell recruitment *in* vivo. In particular, peritonitis was induced in 8 to 12 week-old, female BALB/c mice (Charles River, Orleans, France) by intraperitoneal (i.p.) injection with 200 µl of 0.9% lipopolysaccharide (LPS) free NaCl or 0.25 mg/kg Rantes/CCl5 in 200 µl of LPS free NaCl. To test compound inhibition, doses ranging from 0.5mg/kg to 5mg/kg of a THAP1 chemokine-binding domain/IgG1-Fc fusion constructed as described in Example 18B in 200 µl NaCl were administered i.p.15 min prior to the administration of Rantes (0.25mg/kg i.p.). At 18 hours postinjection, the mice were sacrificed, and the peritoneal cavity was washed three times with 5ml of NaCl and the cells collected were counted with a Neubauer hemocytometer. A second automated cell count was performed using a Beckman cell counter after lysing the red blood cells with a buffer consisting of 150mM NH_4Cl, 0.1mM EDTA, 10mM $KHCO_3$ at pH 7.3. Statistically significant inhibition of *in vivo* cell recruitment was determined by one-way ANOVA, with a Bonferroni post test to compare each treatment with baseline (NaCl). Levels of significance were assigned as follows: $p>0.05$ is not significant; * indicates $p<0.05$, ** indicates $p<0.01$ and *** indicates $p<0.001$.
>
> Figures 25A-C show that the THAP1 chemokine-binding domain/IgG1-Fc fusion inhibits chemokine-induced cell recruitment (chemotaxis). In particular, Figure 25A shows that white blood cell recruitment in mice the presence of Rantes/CCL5 alone is nearly triple that of mice injected with saline only (control mice). Similarly, injection of the human IgG-Fc control did not significantly reduce the level of white blood cell recruitment. However, when mice were injected with the THAP1 chemokine-binding domain/IgG1-Fc fusion prior to the injection of Rantes/CCL5, white cell recruitment was nearly equivalent to that observed for control mice. Figure 25B further shows that mice injected with the THAP1 chemokine-binding domain/IgG1-Fc fusion have similar white blood cell recruitment levels in the presence of Rantes/CCL5 as mice that are injected with anti-Rantes antibody. Figure 25C shows that increasing the concentration of the THAP1 chemokine-binding domain/IgG1-Fc fusion significantly increases the inhibition of cell recruitment.

The ability of the THAP1 chemokine-binding domain/IgG1-Fc fusion to inhibit cell recruitment mediated by other chemokines was tested as described above for Rantes/CCL5 except that in the case of CCL1, recruitment was tested at the CCL1 concentration of 0.5mg/kg.

Figure 26 shows that, in addition to inhibiting *in vivo* cell recruitment mediated by Rantes/CCL5, the THAP1 chemokine-binding domain/IgG1-Fc fusion significantly inhibited *in vivo* cell recruitment in mice injected with CCL1. However, no significant inhibition of cell recruitment was observed in mice injected with CCL2.

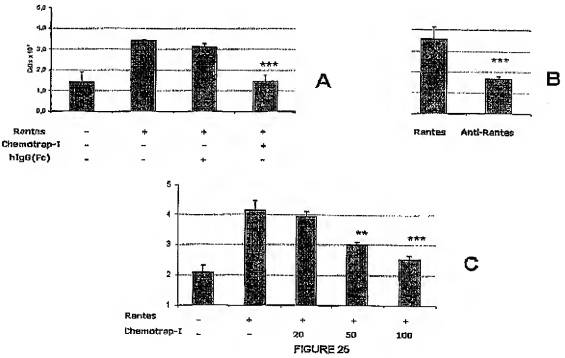


FIGURE 26

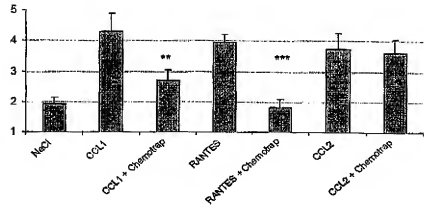


FIGURE 26

9. In the third experiment, which is described in Example 43 of United States Patent Application No. 11/360,450, we demonstrated that the chemokine binding domain of SEQ ID NO: 3 (THAP1 chemokine-binding domain) fused to the constant region of immunoglobulin G (IgG1-Fc) possessed the ability to inhibit the progression of chemokine-induced rheumatoid arthritis (an inflammatory condition mediated by chemokines) *in vivo*. The experimental procedure and results set forth in Example 42 of United States Patent Application No. 11/360,450 are reproduced as follows:

> In this Example, we demonstrate the ability of the THAP1 chemokine-binding domain/IgG1-Fc fusion to block arthritic disease progress was evaluated in the well-characterized collagen antibody-induced arthritis model in mice. In particular, male BALB/c mice (6 wk-old, Harlan Lab., UK) were randomly assigned in groups of 5 animals each. Treatment groups included vehicle (buffer only) negative controls, the THAP1 chemokine-binding domain/IgG1-Fc fusion daily injected i.p. at 5mg/kg in Hepes buffer from Day 2 to Day 9, and dexamethasone (positive control) administered p.o. at 1mg/kg. Experimental arthritis was initially induced on Day 0 of the study by i.v. injection of a four monoclonal antibody cocktail against the CB11 region of chick type II collagen at 100mg/kg, followed about 72 hours later by the intraperitoneal injection of *E coli*

LPS at 2.5mg/kg. Clinical scores and paw volumes of mice from all groups were recorded on day 0, 5, 7 and 9. Clinical scores were assigned using the following scale: 0 = no redness swelling or redness, 1 = mild, but definite redness and swelling of the ankle or apparent redness and swelling limited to individual digits, regardless of the number of affected digits, 2 = moderate redness and swelling of ankle, 3 = severe redness and swelling of the entire paw including digits, and 4 = maximally inflamed limb with involvement of multiple joints. Hind paw thickness was determined with a dial calliper on Day 0, 5, 7 and 9 and presented as mean group values of the average for both left and right hind paws.

Figures 27A-B show that, by day 7, both the group of mice treated with the anti-inflammatory agent dexamethasone and the group treated with the THAP1 chemokine-binding domain/IgG1-Fc fusion exhibited significantly reduced inflammation characteristic of rheumatoid arthritis as compared to mice treated with buffer only (negative control mice).

A

Group No.	TREATMENT	Dose Level (mg/kg)		Arthritic scoring on Study Days: Day 0	Day 5	Day 7	Day 10
1M	Vehicle	0	Mean	0	0.7	1.1	0.1
				(n=5)	(n=5)	(n=5)	(n=5)
			SD	0	0.55	0.84	0.42
2M	Dexamethasone	1	Mean	0	1.1	0.1 +/-	0
				(n=4)	(n=4)	(n=4)	(n=4)
			SD	0	0.68	0.28	0
3M	Chemotmp	5	Mean	0	0.3	0.4 +/-	0
				(n=5)	(n=5)	(n=5)	(n=5)
			SD	0	0.45	0.42	0

*↓ p<0.05 vs. Vehicle control group (1-Way ANOVA Dunnett Multiple Comparison Test)

B

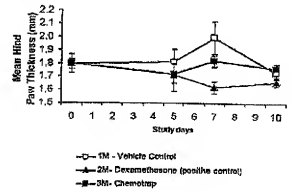


FIGURE 27

10. I declare that the forgoing experimental evidence shows that contacting a chemokine with a chemokine-binding domain of SEQ ID NO: 3 results in the inhibition of chemokine activity.

11. I further declare that all statements made herein of knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

October 30th, 2007
Date

Jean-Philippe Girard, Ph.D.

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EXHIBIT A

CURRICULUM VITAE

Jean-Philippe GIRARD

Date and place of birth : 05/08/1966 in Albi (France)
Nationality : French
Professional address : IPBS-CNRS, 205 Route de Narbonne, 31077 Toulouse, France
Phone : 33 5 61 17 59 67
Fax : 33 5 61 17 59 94
E-mail : Jean-Philippe.Girard@ipbs.fr

Current positions

Research director at INSERM (French NIH)

Deputy-Director of the Institute of Pharmacology and Structural Biology (IPBS-CNRS), a 250-people Research Institute of the CNRS and University of Toulouse (www.ipbs.fr)

Director of the Department of Cancer Biology at IPBS-CNRS

Head of the Laboratory of Vascular Biology at IPBS-CNRS

Co-Founder of ENDOCUBE SAS

Education – Training

June 1989: Biotechnology Engineer of the National Institute of Applied Sciences in Toulouse - (Ranked 1st /36).
September 1989: Master in Biotechnology of the University of Toulouse (Ranked 1st /19).
September 1992 : PhD in Molecular Biology of the University of Toulouse
October 1992 - October 1995: Postoctoral fellow at HARVARD MEDICAL SCHOOL (Center for Blood Research; Boston, USA) in the laboratory of Pr Tim Springer.
March 1996: Permanent researcher position at INSERM (French NIH, Ranked 1st in the national competition CSS2CR21996)
June 2003 : Research director at INSERM (French NIH, Ranked 1st in the national competition CSS2DR22003)

Overall goals of research activity

1) Molecular and functional characterization of high endothelial venules (HEVs), specialized blood vessels for lymphocyte migration, using functional genomic approaches in mouse (HEV-specific KOs), coupled to intravital microscopy analysis. A better understanding of HEVs at the molecular level will allow the development of novel therapeutic approaches for inhibiting HEV development and blocking lymphocyte infiltration into chronically inflamed human tissues (rheumatoid arthritis, Crohn's disease, ...).
2) Functional characterization of novel nuclear factors (chromatin-associated cytokine IL-33, THAP-zinc finger proteins)

Major Research Achievements

1) Purification and molecular characterization of HEV endothelial cells from human tonsils and discovery of HEV-specific genes.

2) Discovery of THAP-zinc finger proteins, a novel family of DNA-binding factors with critical roles in endothelial cell proliferation
3) Discovery of IL-33 (NF-HEV), the most recent addition to the interleukin family, an IL-1-like cytokine associated with chromatin in the nucleus of HEV endothelial cells *in vivo*

Five recent publications

Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, Bouche G and **Girard JP**. (2007). Interleukin-33, the IL-1-like ligand for ST2 receptor, is a chromatin associated nuclear factor in vivo. Proc. Natl. Acad. Sci. USA, 104, 282-287

Cayrol C, Lacroix C, Mathe C, Ecochard V, Loreau E, Lazar V, Dessen P, Mantovani R, Aguilar L and **Girard JP.** (2007). The THAP zinc finger protein THAP1 regulates endothelial cell proliferation through modulation of pRB/E2F cell cycle target genes. Blood, 109, 584-594

Carriere V, Colisson R, Jiguet-Jiglaire C, Bellard E, Bouche G, Al Saati T, Amalric F, **Girard JP** and M'Rini C. (2005). Cancer cells regulate lymphocyte recruitment and leukocyte-endothelium interactions in the tumor-draining-lymph node. Cancer Research, 65, 11639-11648

Clouaire T, Roussigne M, Ecochard V, Mathe C, Amalric F and **Girard JP**. (2005). The THAP domain of THAP1 is a large C2CH module with zinc-dependent sequence-specific DNA binding activity. Proc. Natl. Acad. Sci. USA, 102, 6907-6912

Lacorre DA, Baekkevold E, Garrido I, Brandtzaeg P, Haraldsen G, Amalric F and **Girard JP**. (2004). Plasticity of endothelial cells: rapid dedifferentiation of freshly isolated high endothelial venule (HEV) endothelial cells outside the lymphoid tissue microenvironment. Blood, 103, 4164-4172